GEMINI
BIO-PRODUCTS
800.543.6464 • gembio.com

Continuum Transfection Reagent
(Supplement Protocol for co-transfection)

DNA & siRNA Co-transfection on Adherent Cells

1.1 Cell Seeding

For optimal transfection conditions, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 6-well plates, 150 000 to 250 000 cells are seeded per well 24h prior to transfection. For other culture formats, refer to Table 1 in the standard protocol.

1.2 DNA & siRNA Co-transfection

The following conditions are given per well of a 6-well plate. For other culture format, please refer to the following Supplement Table.

1. Dilute 2 µg of DNA and 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µl of regular high glucose DMEM without serum. Mix by vortexing.
2. Briefly vortex Continuum, and add 2 µl to 6 µl into the diluted DNA & siRNA. Immediately vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently rock the plates back and forth and from side to side, and return the plate to the 37°C CO2 incubator.
6. Analyze after 24 h or later.

NOTE: if you aim to silence the transfected plasmid with the transfected siRNA, then reduce the amount of DNA to 25% of the quantity mentioned above (for example: reduce to 0.5 µg DNA per well of a 6-well plate from 2 µg DNA).

Supplement Table. DNA & siRNA co-transfection guidelines according to the cell culture vessel.

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	DNA (µg)	Continuum (µl)	DMEM (µl)	Growth medium (ml)	Final volume in the well (ml)
24-well	5.5	27.5	0.5 * (0.125)	0.5-1.5	50	0.5	0.55
12-well	11	55	1 *(0.25)	1-3	100	1	1.1
6-well/ 35 mm	22	110	2 *(0.5)	2-6	200	2	2.2
60 mm/ flask 25 cm^2	44	220	4 *(1.0)	4-12	400	4	4.4
100 mm/ flask 75 cm^2	121	605	11 *(2.75)	11-33	1100	11	12.1

*( ) To be used to silence the transfected plasmid with the transfected siRNA